ICU WEAKNESS & OUTCOMES

ICU Weakness: Molecular Mechanisms

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Disclosures

CIHR OCN 126573
CIHR MOP 106545
CIHR MOP 130331
CIHR MOP 140242
MRI Early Researchers Award
Physician Services Incorporate (PSI 0-35)
Stem Cell Network (SCN-72)
Brain-Canada (Z-BRAIN)
Ontario Thoracic Society
McLaughlin Foundation

Tissue Regeneration Therapeutics (TRT)
Northern Therapeutics Inc.
Molecular Mechanisms

- Predominant type II (fast twitch) muscle fibre atrophy
- Selective but patchy loss of myosin filaments
- Non-excitable muscle membrane

Patient Outcomes - Function
7-days, 3, 6, and 12-months post ICU discharge

FIM Total Score

6MWT (% of predicted)

FIM mean scores of 54 at 7\textsuperscript{th} day to 110 at 12 mo post-ICU discharge

60\% of patients were unable to walk at 7 d
Walked distance from 24\% to 75\%\textsubscript{pred} after 12 mo
Rationale

Advance our understanding of the molecular mechanisms that underlie persistent ICU acquired muscle weakness in order to

- Better identify those at risk for long term irreversible sequelae
- To inform effective interventions
Objectives

1) Quantify the degree of skeletal muscle atrophy and functional impairment in a prospective cohort of patients enrolled in Toward RECOVER at 7 days and 6 months post ICU discharge.

2) Perform muscle biopsies (quadriceps femoris) for molecular assessments at 7 days and 6 months post ICU discharge.

3) Determine if these candidate cellular signalling/biologic processes identified in animal models to be critical to the development of ICU acquired muscle dysfunction are activated in humans.

4) Identify signalling networks/markers that are associated with improvement or sustained functional disability.
Study Design

- Inclusion Criteria:

- Pilot study – Nested Cohort Study (Towards RECOVER)

1. Acute presentation to ICU from the community or brief hospitalization (< 1 week) prior to ICU admission
2. Mechanical ventilation for at least 1 week
3. Functional independence prior to ICU admission with no pre-existing neurologic or muscle disease
Study Design

- **Exclusion Criteria:**

1. Immobile prior to ICU admission
2. Pre-existing neurologic or muscle disease
3. Other underlying comorbid disease as documented in the past medical history:
   - e.g. Active hepatitis, post-transplantation, active cancer, decision to move to comfort care,
Study Assessments

- **Functional Assessments:**
  - Skeletal muscle weakness & functional impairment, and atrophy assessed by
    - 6MW, SF-36
    - MRC bedside assessment of muscle power
    - Motor Component of the FIM
    - Quiet postural standing, gait control, isokinetic and strength testing
    - CT mid-thigh quadriceps femoris cross-sectional area (CSA)
    - NCV & EMG to assess for peripheral neuropathy, (functional muscle denervation, dropout of myofibres)
1) Molecular Assessment

- i) Levels and activation of muscle specific signalling molecules
- ii) Cellular localization/redistribution of key molecules
- iii) Morphometric & ultrastructural analyses

2) Explorative Analysis

- Microarray gene expression
- MicroRNA expression
Results

103 Towards RECOVER participants
2 MSICU and 1 Trauma ICU
Sept 2010 – April 2013

2 died
19 excluded
23 missed
32 refused

27 consented

15 - 7 day biopsy
12 - 7 day no biopsy

11 - 6 month biopsy
2 - withdrew
1 – repatriated
1 – medical issues necessitated withdrawal

2 – withdrew
4 – died ICU
2 – medical issues necessitated withdrawal
1 – attempted biopsy unsuccessful
1 - repatriated
2 – withdrew
reasons unknown

15 patients completed 7 day biopsy/assessment
11 completed 6 month biopsy/assessment

dos Santos et al, re-submission, 2015
Persistent muscle weakness correlates with sustained impairment of physical function

FIM motor subscores

% predicted 6MW distance

Quadriceps strength

MRC Sum Score

dos Santos et al, re-submission, 2015
Persistent Atrophy at 6 Months Post-ICU Discharge

dos Santos et al, re-submission, 2015
Dissociation Between Persistent Loss of Muscle Mass and Contractility

dos Santos et al, re-submission, 2015

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>Quads Torque Nm (% pred)</th>
<th>Quads Specific Force (Nm/cm²)</th>
<th>Quads CSA (cm²)</th>
<th>Quads ΔCSA (cm²)</th>
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</thead>
<tbody>
<tr>
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<td>52</td>
<td>M</td>
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<td>1.4</td>
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<tr>
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<td>F</td>
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<td>6.5</td>
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<td>6.1</td>
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</tbody>
</table>

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Interdepartmental Division of Critical Care Medicine

UNIVERSITY OF TORONTO
Muscle protein homeostasis in critical illness

Protein synthesis
- Blunted anabolic signaling
  - Kinase deactivation
- Altered transcriptional regulation
- Compensatory protein synthesis

Protein degradation
- Increased Ca\(^{2+}\)-dependent proteolysis
- Ubiquitin proteasome activation
- Disrupted autophagy

Sepsis, inflammation, cytokines
- Anabolic pathway
  - PISK
  - AKT
  - GSK
  - mTOR
    - p70S6K
    - 4E-BP1
    - eIF-4E
  - MAPK
  - NF-κB
  - FOXO
  - HDAC
  - Transcription factors
  - Myofibrillar mRNA
  - mRNA
  - DNA
  - Muscle genes
  - Protein
  - Ribosome
  - Amino acids
  - tRNA

Proteolytic cleavage
- Ca\(^{2+}\)
- ATP
- ADP
- E2, E3
- Proteasome
- Polypeptides
- Amino acids
- Autophagosome

Resolution of Protein Degradation

dos Santos et al, re-submission, 2015
Absence of Autophagy at 6 months

dos Santos et al, re-submission, 2015
Sarcomeres and Mitochondria at 6 months

Sarcomeric structure

Dos Santos et al, re-submission, 2015
**DISCOVERY**

Clinical and Microarray data

* Functional Independence Measure score
* MRC Sum Score
* Quadriceps muscle mass

DISCOVERY

**Validation**

Modules selected based on:
1) correlation to outcomes of interest
2) Functional & phenotype enrichment
3) TF binding sites enrichment

Module 'informed' genes signature(s)

**Network Analysis**

DAY 7 Post-ICU

Month 6 Post-ICU

ICUAW (11-15 patients)

Healthy controls (8 subjects)

**Gene Set Enrichment Analysis**

ICUAW Gene signature

Independent dataset

GSEA

Co-expression Modules
Differentially expressed genes and gene-sets in ICUAW

Walsh et al. Submitted, 2015
### Modules and Correlation with Phenotypes

<table>
<thead>
<tr>
<th>Module</th>
<th>Correlation with ICUAW</th>
<th>Correlation with phenotypes</th>
<th>GO and HPO terms</th>
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<tbody>
<tr>
<td></td>
<td>Direction</td>
<td>Adjusted P-value</td>
<td>Strength</td>
</tr>
<tr>
<td>M1</td>
<td>-</td>
<td></td>
<td>0.56 (5.5x10^{-3})</td>
</tr>
<tr>
<td>M2</td>
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<td></td>
<td>-0.60 (2.1x10^{-3})</td>
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<td>0.70 (2.5x10^{-4})</td>
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<tr>
<td>M17</td>
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<td>-</td>
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</table>

Gene Expression Data Adjusted for Gender and Age

$R = -0.66$

$p = 3.6 \times 10^{-4}$
Module 1 (Down-regulated Day 7 post ICU discharge)
Module 3 (Up-regulated at Month 6 post-ICU discharge)

- Wound Healing and Repair
  - Connective Tissue Development
  - Extracellular Matrix Deposition
  - Wound Healing
  - Glycosaminoglycan Metabolism
  - Focal Adhesion

- Calcium Handling

- Calcium Handling and Muscle Contraction
  - positive regulation of cytosolic calcium ion concentration
  - calcium ion transport into cytosol
  - cytosolic calcium ion transport

- Regeneration and Differentiation

- Mesenchymal cell development
  - endodermal cell differentiation
  - formation of primary germ layer
  - gastrulation
  - endoderm development
Summary

- Loss of Function (FIMs) correlates with loss of strength
- Loss of strength related to loss of mass or/and contractility
- Dissociation between MASS and CONTRACTILITY
- Ongoing Proteolysis → does not explain persistent muscle loss
- Ongoing Autophagy → does not explain persistent muscle loss
- Mitochondrial structure/number → does not explain ongoing weakness
- Reconstitution of sarcomeric structure
- Correlation between change in Gene expression and Phenotype
- Novel Pathways Associated with Persistent Muscle Weakness
Thank You